portion of the expressed protein (SEQ ID NO:3) is presented below its coding region of nucleotides 971 through 1336.

Figure 5 presents the restriction endonuclease map of WNV capsid protein expression vector pWNVy-DJY.

Figure 6 presents the feature map of WNV capsid protein expression vector pWNVy-DJY.

Figure 7 presents the complete, annotated, double-stranded nucleotide sequence of WNV capsid protein expression vector pWNVy-DJY, having 5864 nucleotide base pairs. Restriction endonuclease sites, features, and translation information for parts of the protein that the construct expresses are indicated in the annotations. The top nucleotide strand is SEQ ID NO:4. The protein sequence of the amino-terminal sIgE leader peptide is presented below its coding region of nucleotides 917 through 970. The protein sequence of the WNV Cp protein portion of the expressed protein is presented below its coding region of nucleotides 971 through 1336.

Figure 8 presents an autoradiograph of electrophoretically resolved, immunoprecipitated, ^{35}S-labeled, *in vitro* transcription/translation products of the two different WNV capsid protein constructs: pWNVh-DJY and pWNVy-DJY. The first lane on the left contains molecular weight markers. The arrow indicates the position of the major *in vitro* translated protein product. The proteins, which are fusions with polyhistidine C-terminal tags, were immunoprecipitated using an anti-His antibody.

Figure 9 shows the complete amino acid sequence of WNV Cp protein (SEQ ID NO:5). The three major histocompatibility (MHC) class II-restricted epitope peptides (WNVC-P1 (SEQ ID NO:6), WNVC-P2 (SEQ ID NO:7), and WNVC-P3 (SEQ ID NO:8)), used in the studies presented herein in Example 3, are shown below the Cp amino acid sequence.

Figure 10 presents the flow cytometry analysis of intracellular IFN-γ expression in *in vitro* stimulated splenocytes from DNA immunized mice. Values presented are the percentage dual positive cells. In the upper panels, the cells were stained for INF-γ and CD44; in the lower panels the cells were stained for CD4 and IFN-γ. The labeling across the top indicates the vector used to immunize the mice plus the stimulus used for the *in vitro* restimulation of the splenocytes. The immunizing vectors were pcDNA3 (pcDNA3.1), pWNVh-DJY (pWNVCh), and pWNVy-DJY (pWNVCy). "No Ag" indicates that the splenocytes were incubated with an *in vitro* translation control (described in Example 3), "protein" indicates that the splenocytes

were incubated with *in vitro* translated Cp protein product from the pWNVy-DJY expression construct.

Figure 11 depicts the results of annexin V flow cytometry analysis of HeLa cells following transfection with enhanced green fluorescent protein (EGFP) expression vector pEGFP2-N1 alone, or in combination with pWNVh-DJY or pWNVy-DJY. Values represent percentage annexin V-positive cells within the EGFP-positive (transfected cells) population.

Figures 12A, 12B, and 12C show the WNV Capsid protein (Cp)-specific antibody response in mice following immunization. Fig. 12A: 100 μg of pCWNVCp expression cassette or control vector was injected intramuscularly at weeks 0, 4, and 8. The sera samples were collected at various days post-immunization and assayed for WNVCp-specific antibody at 1:50, 1:100, 1:200, and 1:400 dilutions. At five months post-immunization, WNVCp-specific antibody responses were detected. The error bars represent the standard deviation of the results from the immunized animals (n=3). Fig. 12B: IgG-subset analysis of WNVCp-specific IgG antibody responses was conducted. WNVCp-specific IgG1 and IgG2a responses examined at 5 months post-immunization as well as the IgG2a/IgG1 ratio are shown. Fig. 12C: WNVCp-specific serum antibody was determined by immunoprecipitation/Western blot analysis. Each immobilized membrane strip was incubated with immune sera from pCWNVCp (W) or pCDNA3 (P). As a positive control, a strip was incubated with an anti-6X His monoclonal antibody (+).

Figure 13 shows production of IFN-γ (Th1), IL-2 (Th1) and IL-4 (Th2) by stimulated T cells. Mice were immunized and their splenocytes were prepared as described in Example 8. The isolated lymphocytes were stimulated for 3 days with WNV Cp pooled peptides. Supernatants were collected and assayed for IFN-γ, IL-2, and IL-4 profiles using ELISA kits. The error bars represent standard deviation (S.D.) values for each experiment.

Figure 14 shows the production of chemokines by stimulated T cells. Mice were immunized and their splenocytes were prepared as described in Example 8. The isolated lymphocytes were stimulated for 3 days with WNV Cp-specific peptide pools. Supernatants were collected and assayed for chemokine profiles using ELISA kits for RANTES and MIP-1β. The error bars represent standard deviation (S.D.) values of each experiment.

Figures 15A and Figure 15B show the induction of positive antigen-specific CTL response. Fig. 15A: Splenocytes from immunized mice were tested for CTL response using target cells treated with pooled WNV Capsid peptides. Fig15B: Supernatants from effectors

stimulated for CTL assay were collected at day five and tested for IFN-γ production. The error bars represent standard deviation (S.D.) values for each experiment.

Figures 16A, 16B, and 16C show the analyses of muscle tissue. Frozen muscle sections were prepared from DNA injected animals and stained with hematoxylin and eosin (H&E) stain. Slides from pCDNA3 (control) immunized mice (Fig. 16A) and pCWNVCp immunized mice (Fig. 16B) are shown. The panels shown are at 40X magnification. Fig. 16C: Identity of the muscle infiltrating cells in pCWNVCp immunized mice. The cells were harvested as described in Example 11, and were identified by FACS using antibodies to CD4, CD8, Mac3, CD11c, CD86, and B220.

Figure 17 shows the alignment of WNV Cp protein sequence with portions of the sequences of capsid proteins from other *Flaviviruses*. The top comparison is between the first 123 amino acids of Cp protein from Kunjin virus (KJV; GenBank accession number BAA00176 (gi:221967), which is incorporated herein by reference) (SEQ ID NO:9) and the complete 123 amino acid sequence of WNV Cp protein. The middle comparison is between the first 113 amino acids of Cp protein of a Japanese encephalitis virus (JEV; GenBank accession number NP_059434 (gi:9626461), which is incorporated herein by reference) (SEQ ID NO:10) and the first 114 amino acids of WNV Cp protein (SEQ ID NO:11). The bottom comparison is between amino acids from an internal portion of the Cp protein of a Dengue virus (DEN2; GenBank accession number AAG30730 (gi:11119732), which is incorporated herein by reference) (SEQ ID NO:12) and amino acids 10 through 98 of WNV Cp protein (SEQ ID NO:13). This alignment required looping out of a lysine (K appearing above the line) from the stretch of amino acids LTKR in the DEN2 sequence. The values in brackets are identity/homology scores, where a maximum possible score is 590. Comparisons and alignments were generated by MacVector.

Figure 18 shows the alignment of the WNV Cp protein sequence with portions of the sequences of proteins from other viruses and with portions of the sequences of proapoptotic proteins. The complete sequence of the WNV Cp protein (amino acids 1 - 123) appears at the top in bold. Shown are 6 comparisons of WNV Cp with other viral proteins and 5 comparisons of WNV Cp with proapoptotic proteins. The viral protein comparisons are as follows: 1) amino acids from an internal portion of Human Immunodeficiency Virus-1 (HIV-1) 89.6 Vpr protein (GenBank accession number AAA81039 (gi:1055033), which is incorporated herein by reference) (SEQ ID NO:27) and amino acids 68 through 110 of WNV Cp protein (SEQ ID NO:28); 2) amino acids from an internal portion of Herpes Simplex Virus major capsid protein